若年者精巣上体炎患者におけるクラミジアトラコマチス検出法としての新しいPolymerase chain reaction法の臨床的検討

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CLINICAL EVALUATION OF A NEW POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF *CHLAMYDIA TRACHOMATIS* IN YOUNG PATIENTS WITH ACUTE EPIDIDYMITIS

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Specimens from 15 young patients presenting with acute epididymitis were tested for the presence of *Chlamydia trachomatis* by an enzyme immunoassay (EIA), polymerase chain reaction (PCR), and for other bacteria by standard laboratory techniques. *C. trachomatis* urethral infection was detected in 3 patients by an EIA test of the urethral swabs (20%) and in 13 patients by the PCR (87%). This difference in detection rate was statistically significant ($p<0.005$). Thir-teen specimens were positive by the PCR, but only three of them were positive by the EIA method. These findings indicate that the PCR assay is a highly sensitive assay for the detection of *C. trachomatis* in male urine specimens and provides a noninvasive technique for routine screening of chlamydia infection in the patient with acute epididymitis.

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Key words: *Chlamydia trachomatis*, PCR, EIA, Epididymitis

INTRODUCTION

Acute epididymitis in men who are younger than 35 years of age who have no functional or anatomical abnormalities of the urinary system is usually caused by Chlamydia trachomatis (*C. trachomatis*)[1]. However, many affected patients have no history of a urethral discharge or clinical evidence of urethral inflammation. For these reasons, men with acute epididymitis are often treated inappropriately and treatment of the patient's sexual partner for chlamydial cervicitis is often not pursued. In a high proportion of these cases, identification of *C. trachomatis* has been based solely on the antigen detected by enzyme immunoassay (EIA) or raised antibody titers. This still leaves a majority of patients in which no infectious etiology can be found. One reason for this may be the relative insensitivity of conventional laboratory methods used for the detection of chlamydia infection. While these tests usually require an urethral swab, recent studies have evaluated the reliability of enzyme immunoassays in detecting chlamydia in the urine[2].

Recently the polymerase chain reaction (PCR) has been applied for detection of *C. trachomatis* in urethritis[3,4] and cervicitis[5] and its usefulness has been testified. More recently a new noninvasive and sensitive diagnostic test to evaluate male urine specimens (Amplicore *C. trachomatis;* Roche Molecular Systems, Branchburg, N.J.) has been developed. It combines the technique of the PCR and colorimetric microwell DNA hybridization for the detection of *C. trachomatis* in first-void male urine specimens. In this communication, we compared the PCR method with the EIA in the detection of *C. trachomatis* in young patients presenting with acute epididymitis.

MATERIALS AND METHODS

Fifteen patients (age range 18-34 years) were included in this study. Epididymitis was a clinical diagnosis made on the basis of clinical history and the finding of a tender swollen epididymis on physical exami-

nation. Objective evidence of urethritis was diagosed when there were more than 5 polymorphonuclear leukocytes per high powered field in the urethral swab specimen. First void urine sample and urethral swab were collected for detection of *C. trachomatis*. The urethral swab was obtained using fine, cotton-tipped swabs inserted 3 to 4 cm into the urethra. Urethral swabs were Gram stained and examined under a microscope. The antigen of *C. trachomatis* from the urethral swab specimen was detected by enzyme immunoassay (Chlamydiazyme™). The polymerase chain reaction (PCR) for detection of *C. trachomatis* was performed on DNA extracted from the urine sample. It was performed using a commercial kit of PCR for detection of *C. trachomatis* (Amplicore *C. trachomatis;* Roche Molecular Systems, Branchburg, N.J.)[6]. Routine urinary culture for other bacteria was also performed. Serum IgG and IgA antibodies for *C. trachomatis* was measured by the ELISA kit, HITAZYME (HITAZYME, Hitachi Chemical).

The chi-square test was used to compare the detection rate in each investigational method for *C. trachomatis*. Differences were considered significant at $p<0.05$.

RESULTS

A total of fifteen patients attending our urology clinic were evaluated for *C. trachomatis* infection by a new PCR assay for urine specimens, and the results were compared with those obtained by the EIA method for urethral swab specimens (Table 1). Thirteen specimens were positive by the PCR, but only three were positive by the EIA method. Only two specimens were negative by both tests. There were 10 positive results by the PCR and negative by the EIA method. There was no patient with a negative result by the PCR but positive result by the EIA method. The detection rate of *C. trachomatis* by the PCR method was 87%, while that by the EIA method was only 20%. This difference was statistically significant ($p<0.005$). There were two *Escherichia coli* strains and a *Proteus mirabilis* strain found in the urine culture from the patient positive by the PCR, but negative by the EIA method. There was one *E. coli* strain found from the patient positive by both PCR and EIA methods (Table 1). In these four patients, culture of expressed prostatic secretion (EPS) was negative and the leukocyte value in the EPS was 2~5/hpf. There was no tenderness of prostate in

Table 1. Results of tests to detect microorganisms in specimens from 15 patients with acute epididymitis

Patient no.	Age	Duration of symptoms (days)	Tests for C.trachomatis		Tests for other bacteria	
			PCR	EIA	Urine	Urethra swabs
1	25	7	−	−	−	−
2	18	2	+	−	E.coli	−
3	28	5	+	−	−	−
4	34	9	+	−	E.coil	−
5	22	3	+	+	−	−
6	34	4	+	+	E.coil	−
7	26	7	+	+	−	−
8	21	2	+	−	−	−
9	32	3	+	−	Proteus mirabilis	−
10	34	5	+	−	−	−
11	25	2	−	−	−	−
12	26	3	+	−	−	−
13	29	5	+	−	−	−
14	32	3	+	−	−	−
15	28	4	+	−	−	−

these patients. No bacteria were found in the gram stained specimens of the urethral swab under a microscope (Table 1). The IgA antibody was detected in the serum in all patients.

DISCUSSION

C. trachomatis infection is the most prevalent sexually transmitted bacterial infection in Japan[1] and is the most important cause of nongonococcal urethritis and acute epididymitis in men under 35 years of age[7]. It is important to note that from 10% to 25% of infected men may be asymptomatic[8]. Chlamydia infections are capable of being invasive, resulting in epididymorchitis, testicular atrophy and ductal obstruction[9]. Therefore, screening for these infections is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections and to prevent future possible infertility, but also to identify asymptomatic infected males who serve as reservoirs for *C. trachomatis* disease.

Traditionally, the gold standard for the identification of *C. trachomatis* infections is tissue culture of the urethral swab. This technique is invasive, time-consuming and labor-intensive. It takes 3 to 6 days to complete, and it requires access to specialized facilities and trained personnel. Other non-culture-based immunologic techniques have been developed in an effort to detect *C. trachomatis* in noninvasive samples One such test is the EIA method to detect *C. trachomatis* by using first-catch urine[10]. This technique is relatively fast and easy to complete, but the sensitivity of the test for urine specimens from asymptomatic men remain relatively low (62.1 to 79.1% for the Abbott Chlamydiazyme test)[10].

The primers-directed enzymatic amplification of DNA[11], PCR, has been shown to offer a number of potential advantages for genetics, microbiology, oncology and so on. PCR has been applied for diagnosis of viral[12,13] and bacterial infections[14,15]. The system for detection of *C. trachomatis* using PCR has been reported in urethritis[3,4] and cervicitis[5] and demonstrated to be as useful as the culture method[14] and enzyme-immunoassays[16]. However, it has not been extensively studied for use with male urine specimens. When a commercially available DNA amplification kit (Genemed Biotechnologies, San Francisco, CA) was used to compare the PCR, EIA, and culture, the sensitivities of both the PCR and EIA were approximately 90%, for male urine compared with symptomatic and asymptomatic urethral culture[17,18]. Overall, for both males and females, the PCR was more sensitive (95.6%) than the EIA (87%). Specificities were 97.7% (99.4%, for men and 95.7% for women) for the EIA and 98% (99.4% for men and 96.5% for women) for the PCR[17].

The Roche Amplicor *C. trachomatis* method combines a PCR and colorimetric detection for the detection of *C. trachomatis* in first-void male urine specimens. Overall, it is a relatively easy method to process a high volume of specimens (n=90) in approximately 4.5 h[6]. In this PCR method, the primers are derived from the cryptic plasmid, yielding a 207-bp DNA fragment[6]. In our present study using this PCR technique, it showed a high detection rate of *C. trachomatis* in urine samples compared with the conventional EIA method for urethral swabs. This difference can be explained by the difference in sensitivity between the two methods. Another possible explanation for the low detection rate with the urethral swab is that it is not a sufficient method for obtaining specimen from entire urethra in which *C. trachomatis* is colonized.

CONCLUSION

Medical treatment of the young patient with acute epididymitis should be based on the specificity and positive predictive value of an assay for the detection of *C. trachomatis* often causing acute epididymitis. The reliability of the PCR assay for the detection of *C. trachomatis* promptly provides urologists a clear indication for initiation of adequate treatment. PCR analysis of urine is a highly sensitive and specific noninvasive technique for the diagnosis of Chlamydia infection and can be used for the early identification of the infected

patient with acute epididymitis caused by *C. trachomatis*.

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和文抄録

若年者精巣上体炎患者におけるクラミジアトラコマチス検出法としての新しい Polymerase chain reaction 法の臨床的検討

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15名の若年者精巣上体炎患者からえられた尿および尿道スワブに存在するクラミジアトラコマチスを検出する目的で，免疫酵素抗体法（EIA）と polymerase chain reaction 法（PCR）を行った．他の一般細菌の検出には，尿培養と尿道スワブの顕微鏡検査を施行した．クラミジアトラコマチスによる尿道炎は EIA 法ではわずかに 20％に認められたのみであったが，PCR 法では87％の患者に認められた．この差は統計学的に有意な差であった（$p<0.005$）．13例の PCR 陽性例のうち EIA 法でも陽性と判定された症例はわずかに 3 例のみであった．以上の結果より PCR 法は男性の尿中におけるクラミジアトラコマチス検出においては，感受性が高く，急性精巣上体炎患者におけるクラミジア感染症の非侵襲的なスクリーニング法として有用であると思われる．

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